

What is claimed is:

1. A three-dimensional optical method for determining the volume, V, and hemoglobin content, HC, of individual red blood cells, said method comprising the steps of;

- a) treating an anti-coagulated whole blood sample with a reagent solution, said solution comprising a sphering agent and a neutrally buffered isotonic saline solution;
- b) passing a red blood cell isolated from said sample through a light beam directed along an optical path at a selected wave length;
- c) measuring the resultant magnitude of a first forward angle light scatter signal, a second intermediate angle light scatter signal, and a third side-angle light scatter signal from each cell;
- d) projecting a three-dimensional coordinate of said light scatter signals from each cell onto a precalibrated three dimensional surface containing grid lines of V and HC;
- e) determining the values of V and HC by the location of each projected intercept onto said three dimensional grid surface.

2. The method of claim 1, whereby said scatter signals are determined by the volume and hemoglobin concentration of each cell, where said hemoglobin concentration is determined as a function of the index of refraction of hemoglobin at wavelength of said beam of light.

3. The method of claim 1 wherein said selected wavelength corresponds to visible light in a range of 400 nm to 800 nm.

4. The method of claim 1, whereby said three-dimensional surface is calculated based on Mie scatter theory, and is a function of the angles of said three signals relative to the angle of said light beam, the wavelength of said light beam, and the refractive index of hemoglobin at said wavelength.

5. The method of claim 1, whereby said reagent solution further contains a nucleic acid stain which enables separation of reticulocytes from mature red blood cells in said blood sample by means of a fourth fluorescence signal.

5 6. The method of claim 5, which further includes a red blood cell gate for excluding other cellular particles including white blood cells, platelets, and nucleated red blood cells, said gate established by constructing a two-dimensional cytogram of forward scatter and fluorescence.

7. The method of claim 5, which further includes a red blood cell gate for excluding other cellular particles including white blood cells, platelets, and nucleated red blood cells, said gate established by constructing a two-dimensional cytogram of light loss and fluorescence.

8. The method of claim 5, wherein said three dimensional surface grid method is applied to determine volume and hemoglobin concentration of said reticulocytes.

9. The method of claim 1, which includes the identification of abnormally shaped red blood cells by determining the closest distance of the cell point from said three-dimensional grid surface measured in the direction normal to said grid surface.

10. The method of claim 1, wherein one is able to quantify the percent of macrocytes, microcytes, hypochromic cells, hyperchromic cells, or combinations thereof in said blood sample from the bivariate distribution of individual cell volume and hemoglobin concentration of said sample.

11. The method of claim 1, which includes continuous monitoring of system standardization by determining the degree of symmetry of the cell population distances from the three-dimensional grid surface.

12. A three-dimensional optical method for determining the volume, V, and hemoglobin content, HC, of individual red blood cells, said method comprising the steps of;

- a) treating an anti-coagulated whole blood sample with a reagent solution, said solution comprising a sphering agent and a neutrally buffered isotonic saline solution ;
- b) passing a red blood cell isolated from said sample through a light beam directed along an optical path at a selected wavelength;
- c) measuring the resultant magnitude of one forward angle light scatter signal, one light loss signal, and a third side-angle light scatter signal from each cell;
- d) projecting a three-dimensional coordinate of said light scatter signals from each cell onto a pre-calibrated three dimensional surface containing grid lines of V and HC;
- e) determining the values of V and HC by the location of each projected intercept onto said three dimensional grid surface.

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~~13.~~ An apparatus for simultaneously determining white blood cell and red blood cell differentiation comprising:

- a) a means for directing a beam of light along an optical path;
- b) a means for passing a cell through said light beam to produce light scattering patterns of desired angular intervals;
- c) an optical detector with multiple discrete regions, corresponding to predefined angular intervals;
- d) electronic pre-amp means for selectively isolating desired angular intervals for said white blood cell and said red blood cell differential analysis, respectively;
- e) a means for generating, concurrently, signals from each angular interval, corresponding to the intensity of the scattered light within said angular intervals, respectively; and
- f) a means for determining the volume and hemoglobin concentration on cell by cell basis of said red blood cells, and size and complexity of said white blood cells.

15/ 14. The apparatus of claim ~~13~~¹⁴, wherein said determining step includes passing a plurality of said red blood cells, one at a time, through said beam of light, such that each of said red blood cells develops at least a 1st forward angle light scatter signal, a 2nd intermediate angle light scatter signal, and a 3rd side angle light scatter signal to determine the volume, hemoglobin concentration, and cell shape abnormality.

17/ 15. The apparatus of claim ~~13~~¹⁴, wherein said determining step includes passing a plurality of said red blood cells, one at a time, through said beam of light, such that each of said red blood cells develops at least one light loss signal, a 2nd intermediate angle light scatter signal, and a 3rd side angle light scatter signal to determine the volume, hemoglobin concentration, and cell shape abnormality.

15/ 16. The apparatus in claim ~~14~~¹⁵, wherein a further determining step includes means for developing a 4th concurrent fluorescent signal of said red blood cells for determining maturity of each cell.

18/ 17. The apparatus of claim ~~13~~¹⁴, wherein the determining step includes passing a plurality of said white blood cells, one at a time, through said beam of light, such that each of said white blood cells develop at least a 1st forward angle light scatter signal, a 2nd intermediate angle light scatter signal, a 3rd side angle polarized light scatter signal, and a 4th side angle depolarized light scatter signal to determine the size, cell internal complexity, and granularity of each cell.

19/ 18. The apparatus of claim ~~13~~¹⁴, herein the determining step includes passing a plurality of said white blood cells, one at a time, through said beam of light, such that each of said white blood cells develop at least one light loss signal, a 2nd intermediate angle light scatter signal, a 3rd side angle polarized light scatter signal, and a 4th side angle depolarized light scatter signal to determine the size, cell internal complexity, and granularity of each cell.

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19. The apparatus of claim 13, wherein said light beam is selected from the visible spectrum in a range of 400 nm to 800 nm.

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20. The apparatus of claim 19, wherein said light source is selected from the group consisting of:

- a) an argon-ion gas laser at 488 nm;
- b) a solid state laser at 532 nm; and
- b) a helium-neon gas laser at 632 nm.
- c) a red diode laser
- d) a green diode laser
- e) a blue diode laser

13/
21. The method of 1 or 12, wherein said sphering agent is a nonionic surfactant.